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Chromosome Position: Now, Where Was I?

Dispatch

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Is the nuclear organisation of chromosomes inherited through mitosis, when the nuclear membrane is broken down, and is it propagated to the nuclei of daughter cells? Two recent studies address this question using similar live cell imaging techniques, but reach different conclusions.

The organisation of chromosomes within the interphase nucleus is implicated in the regulation of gene expression and in generating chromosome translocations [1]. During mitosis in vertebrate cells, however, the most prominent landmarks of nuclear structure, the nuclear envelope and the nucleolus, are broken down. Chromosomes condense and congress to a flat metaphase plate which lacks the obvious three-dimensional spatial organisation of the nucleus. A key question is therefore how chromosomal order is re-established in daughter nuclei after mitosis. Two recent papers [2,3] address to what extent the nuclear position of chromosomes is transmitted through mitosis, and although they use similar experimental approaches the conclusions that they reach are rather different.

Chromosomes are not randomly arranged in the vertebrate nucleus. A radial organisation of chromosome territories has been described, with gene-poor chromosomes located in a zone close to the nuclear edge and gene rich-chromosomes in the centre of the nucleus [1]. This does not mean that chromosomes must have a precise spatial relationship to each other: each chromosome need not have reproducible neighbours. Indeed, several studies indicate that chromosome neighbourhoods vary from cell to cell [4,5]. Also, the analysis of translocations recovered in cells exposed to ionising radiation supports a clustering of certain human chromosomes in the centre of the nucleus, but does not indicate any other consistent chromosome neighbourhoods [6]. Nevertheless, there have been some reports of preferential chromosome positioning [7].

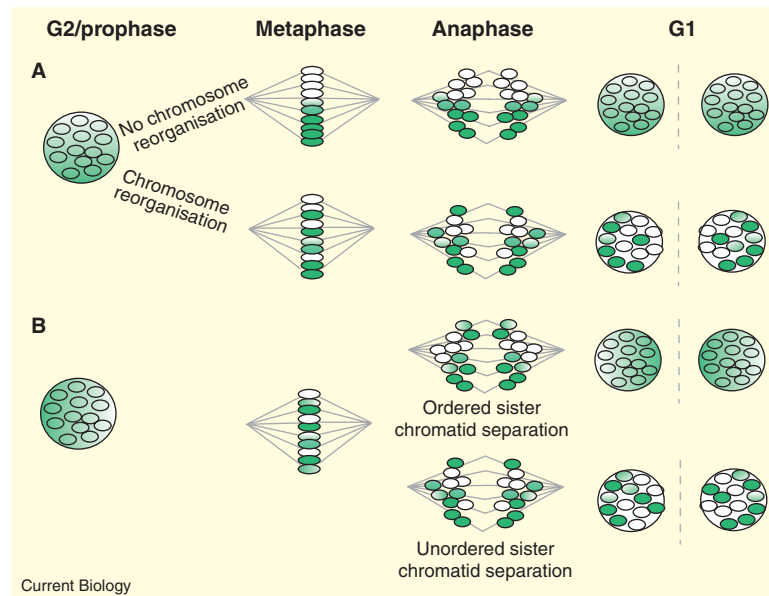
The inheritance of interphase chromosomal order was considered by Boveri in the early 20th century, on the basis of his studies of nematode chromosomes [3,8]. Boveri proposed that chromosome organisation was stably maintained during interphase, altered during congression of chromosomes to the metaphase plate, and the new order then propagated through anaphase and telophase to two rather symmetrical daughter nuclei. An alternative view is that the spatial information about chromosome positioning is inherited from the mother nucleus via the arrangement of chromosomes with respect to the mitotic spindle and

metaphase plate. An important prediction of the second hypothesis, but not of the first, is that the arrangement of chromosomes in daughter nuclei should resemble that of the mother nucleus.

The recent studies [2,3] have used 21st century fluorescence live cell imaging techniques to ask if chromosome position in mammalian cells can be inherited from a mother nucleus, through the events of mitosis, and into the two daughter nuclei. To follow chromosomal domains in living cells, both groups employed cell lines expressing histone H2B tagged with GFP [3], or its spectral variants CFP and YFP [2], relying on the observation that most H2B exchanges in and out of chromatin quite slowly ($t_{1/2} \geq 2$ hours) [9]. Areas of chromatin were photobleached in nuclei prior to mitosis, then the bleach pattern followed through to the daughter nuclei. One group [3] compared bleached and unbleached H2B–GFP signals, whereas the other [2] photobleached H2B–YFP, leaving the H2B–CFP unbleached as a reference.

In the first case, substantial re-organisation of chromosomes was noted as cells progressed from G2 into metaphase [3]. In 13 out of 20 cells, a single region of unbleached chromatin at the edge of one pole of the nucleus became redistributed to dispersed parts of the metaphase plate, suggesting that re-ordering of chromosomes had occurred during prophase and prometaphase (Figure 1A). In about 40% of cases, the unbleached chromatin was then clustered together toward one pole of the resultant daughter nuclei, restoring some of the arrangement of chromatin that had been in the mother nucleus, but with evidence of local rearrangements. Another 30% of daughter nuclei had clusters of unbleached chromatin, but also remote patches of unbleached chromatin. In a further 25% of cases the unbleached chromatin, which had been located together in the mother nucleus, was found distributed over large parts of the daughter nuclei. From these observations, Walter *et al.* [3] conclude that the positions of chromosome segments in daughter nuclei differ significantly from the positions in the mother cell nuclei and therefore that “significant changes of chromosome territory order occur during mitosis”.

Gerlich *et al.* [2], in contrast, conclude that the organisation of chromatin in daughter nuclei has a “strong similarity” to that in the mother cell nucleus. and thus that “chromosome positions are heritable through the cell cycle”. Moreover, by analysing bleach zones in different orientations with respect to the mitotic spindle (Figure 1) they propose separate mechanisms through which conservation of nuclear organisation is mediated. It is suggested that, in the plane of the metaphase plate, perpendicular to the spindle axis, chromosomes congress onto the plate without substantial re-organisation. Subsequent anaphase/telophase movements result in mirror symmetrical daughter nuclei (Figure 1A). What is harder to envisage is how the spatial organisation of chromosomes parallel to the spindle axis can be



to the spindle axis. Spatial organisation in this dimension will be lost on the flat metaphase plate, but if there is ordered separation of sister chromatids, allowing specific chromosomes to move poleward before others, then the nuclear organisation of the mother nucleus can be restored in the daughters.

transmitted through an essentially flat metaphase plate (Figure 1B). Chromosome-specific differences in the timing of sister chromatid separation are suggested to underlie this preservation of spatial organisation, such that certain chromosomes reproducibly separate and begin anaphase movements toward the spindle poles before others. Early separating chromosomes will end up on the side of the nucleus furthest from the cleavage furrow (Figure 1B).

There is a precedent for this mechanism, as heterochromatin appears to be essential for maintaining sister chromatid cohesion, up until the destruction of cohesin at the metaphase-anaphase transition [10]. In mammalian cells, the chromosomes with the largest blocks of pericentric heterochromatin are the last to separate, and this is altered if heterochromatin structure is perturbed by the dye Hoechst 33258 [11]. Similarly Gerlich *et al.* [2] found that Hoechst 33258 randomises chromosome position along the spindle axis.

How can the apparently contradictory findings of these two studies be reconciled? One possibility is that it is merely a matter of degree. Walter *et al.* [3] conclude that mitosis allows substantial reorganisation of global nuclear order, but 40% of their bleaching experiments show the retention of neighbouring chromatin domains from one cell cycle to the next. Gerlich *et al.* [2] conclude that global chromatin order is inherited from one cell cycle to the next, yet mixing between bleached and unbleached domains is apparent in their daughter nuclei. Differences in cell type, rat NRK cells versus human HeLa cells, may be another factor.

Another possibility is that the two groups analysed the mother nuclei at different periods before mitosis, and the daughter G1 nuclei at different periods after metaphase. The images shown by Gerlich *et al.* [2] suggest that the cells were already in prophase when they were bleached, whereas in Walter *et al.* [3] the

Figure 1. Tracking nuclear organisation of chromosomes during mitosis.

Chromatin tagged with H2B-GFP/YFP (green) is photobleached (white) in part of the nucleus before mitosis. Bleached and unbleached regions are then followed through chromosome congression onto the mitotic spindle (grey) forming the metaphase plate, and then through sister chromatid separation and anaphase movements through to formation of the daughter G1 nuclei. The dashed grey line indicates the position of the spindle midzone/cell cleavage body. (A) Chromatin is bleached along the axis of the metaphase spindle (perpendicular to the metaphase plate). If little chromosome reorganisation occurs during congression, then the daughter nuclei will resemble both each other and the mother nucleus. If chromosome reorganisation does occur during congression, then the daughter nuclei may resemble each other, but will have different chromosome organisation than the mother nucleus. (B) Chromatin is bleached parallel

bleaching was done before chromosome condensation, thus allowing for more post-bleach chromosome reorganisation during prophase. Daughter nuclei were then analysed up to 90 minutes after metaphase in one paper [3], but for only 30–60 minutes in the other [2]. Whilst there might be a preservation of order at very early stages of G1, this could be eroded by chromatin re-organisation as G1 progresses.

The analysis of chromatin dynamics in mammalian cells by many groups has shown that chromatin has only limited mobility during most of interphase, and indeed this was confirmed in both of the papers discussed here. Some data, however, suggest that there can be quite dramatic chromosome movements and re-organisation during the first 1–2 hours of G1 [12–14]. Changes in chromatin position of several microns in early G1 were also described in one of the present studies [3].

Both studies suggest that a significant level of global chromosomal order is established at the time of chromatid separation. But the use of fluorescent *in situ* hybridisation (FISH) [3] to look at the organisation of chromosomes in two and four cell clones indicated that, although symmetrical arrangements of chromosomes may exist after a single mitosis, this order is eroded by subsequent cell divisions. A substantial inheritance of chromosomal order during metaphase-telophase, diluted by enhanced chromosome mobility during prometaphase and early G1, is a compromise conclusion for both studies.

One shortcoming of both the analyses described here is that there is no way of telling exactly which chromosome domain is being analysed; so analysing the behaviour of more defined chromosomal loci may help [15]. One testable prediction of the chromatid separation model is that human chromosomes 1, 16, Y and the acrocentric chromosomes should end up on

the side of the nucleus closest to the cleavage furrow, since these chromosomes have large blocks of pericentric heterochromatin, and are the last to separate at anaphase [16].

What are the functional implications of inheriting some degree of chromosomal order through mitosis? It could be important in setting up a functional nuclear architecture, but if chromatin domains can move several microns in early G1, almost any locus should be within reach of a nuclear structure, such as the nuclear envelope, of a typical human nucleus, before more constrained chromatin motion preserves organisation during the rest of interphase. This might enable the right balance of inheritance of (epigenetic) information and plasticity to be attained. Analysing the dynamics of individual defined loci during early G1 will be important in assessing whether this is the case.

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